H36.D2.B7 ANTI-TISSUE FACTOR LIGHT CHAIN VARIABLE REGION

GACATTCAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTCACCATCACATGC
D I Q M T Q S P A S Q S A S L G E S V T I T C

CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC
L A S Q T I D T W L A W Y Q Q K P G K S P Q L

CTGATTTATGCTGCCACCAACTTGGCAGATGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGCACA
L I Y A A T N L A D G V P S R F S G S G S G T

AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAAATTATT TACTGTCAACAAGTTTAC
K F S F K I S S L Q A E D F V N Y Y C Q Q V Y

AGTTCTCCATTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA
S S P F T F G A G T K L E L K

FIG. 1A

H36.D2.B7 ANTI-TISSUE FACTOR HEAVY CHAIN VARIABLE REGION

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGCAGGTATCCTGCAAG
E I Q L Q Q S G P E L V K P G A S V Q V S C K

ACTTCTGGTTACTCATTCACTGACTACAACGTGTACTGGGTGAGGCAGAGCCATGGAAAGAGCCTTGAG
T S G Y S F T D Y N V Y W V R Q S H G K S L E

TGGATTGGATATATTGATCCTTACAATGGTATTACTATCTACGACCAGAACTTCAAGGGCAAGGCCACA
W I G Y I D P Y N G I T I Y D Q N F K G K A T

TTGACTGTTGACAAGTCTTCCACCACAGCCTTCATGCATCTCAACAGCCTGACATCTGACGACTCTGCA
L T V D K S S T T A F M H L N S L T S D D S A

GTTTATTTCTGTGCAAGAGATGTGACTACGGCCCTTGACTTCTGGGGCCAAGGCACCACTCTCACAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V

TCCTCA
S S

FIG. 1B

ANTIBODY	APPARENT K_1, M^{-1}	APPARENT K_1, M
BY ELISA		
D2	5.2×10^9	1.9×10^{-10}
I47	6.5×10^9	1.5×10^{-10}
K73	9.8×10^9	1.0×10^{-10}
K80	2.3×10^9	4.3×10^{-10}
L102	2.5×10^9	4.0×10^{-10}
L133	1.7×10^9	5.9×10^{-10}
BY BIACore		
H36	3.1×10^{10}	3.2×10^{-11}
I43	2.3×10^9	4.3×10^{-10}
I47	3.2×10^9	3.1×10^{-10}
L133	4.6×10^9	2.2×10^{-10}
M107	1.1×10^9	9.1×10^{-10}

FIG. 2

ANTIBODY NAME	% INHIBITION ANTIBODY PREINCUBATED WITH TF/VIIa
D1	0
D1B	1
H31	4
H36	95
I43	1
J131	7
K80	0
K82	0
K87	1
L97B	7
L101	0
L102	0
L105	0
L133	0
M5	1
M107	34

FIG. 3

ANTIBODY NAME	% INHIBITION TF PREINCUBATED WITH ANTIBODY PRIOR TO ADDITION OF VIIa	% INHIBITION TF PREINCUBATED WITH VIIa PRIOR TO ADDITION OF ANTIBODY
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
I43	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

FIG. 4

[rhTF], nM	[H36.D2], nM	H36.D2/rhTF MOLAR RATIO	CLOTTING TIME (SECONDS)	% INHIBITION OF rhTF FUNCTION
0.0048	0 1.61 3.23	0 335.4 670.8	102.3 114.3 121.3	0 31.3 45.8
0.023	0 1.61 3.23 6.45	0 70.0 140.0 280.4	77.6 85.3 91.1 99.6	0 52.2 65.2 73.9
0.092	0 3.23 6.45 12.90	0 35.1 70.1 140.2	49.3 65.8 88.5 113.3	0 65.2 90.2 95.7
0.46	0 6.45 12.90 32.30	0 14.0 28.0 70.2	32.6 52.7 80.2 117.9	0 82.4 96.7 99.3
2.30	0 16.10 32.30 64.50	0 7.0 14.0 28.0	23.9 47.1 95.2 115.3	0 94.4 99.7 99.9
11.52	0 16.10 32.30 64.50 161.30	0 1.4 2.8 5.6 14.0	22.2 30.2 46.0 87.6 114.0	0 93.4 98.8 99.9 100.0

FIG. 5

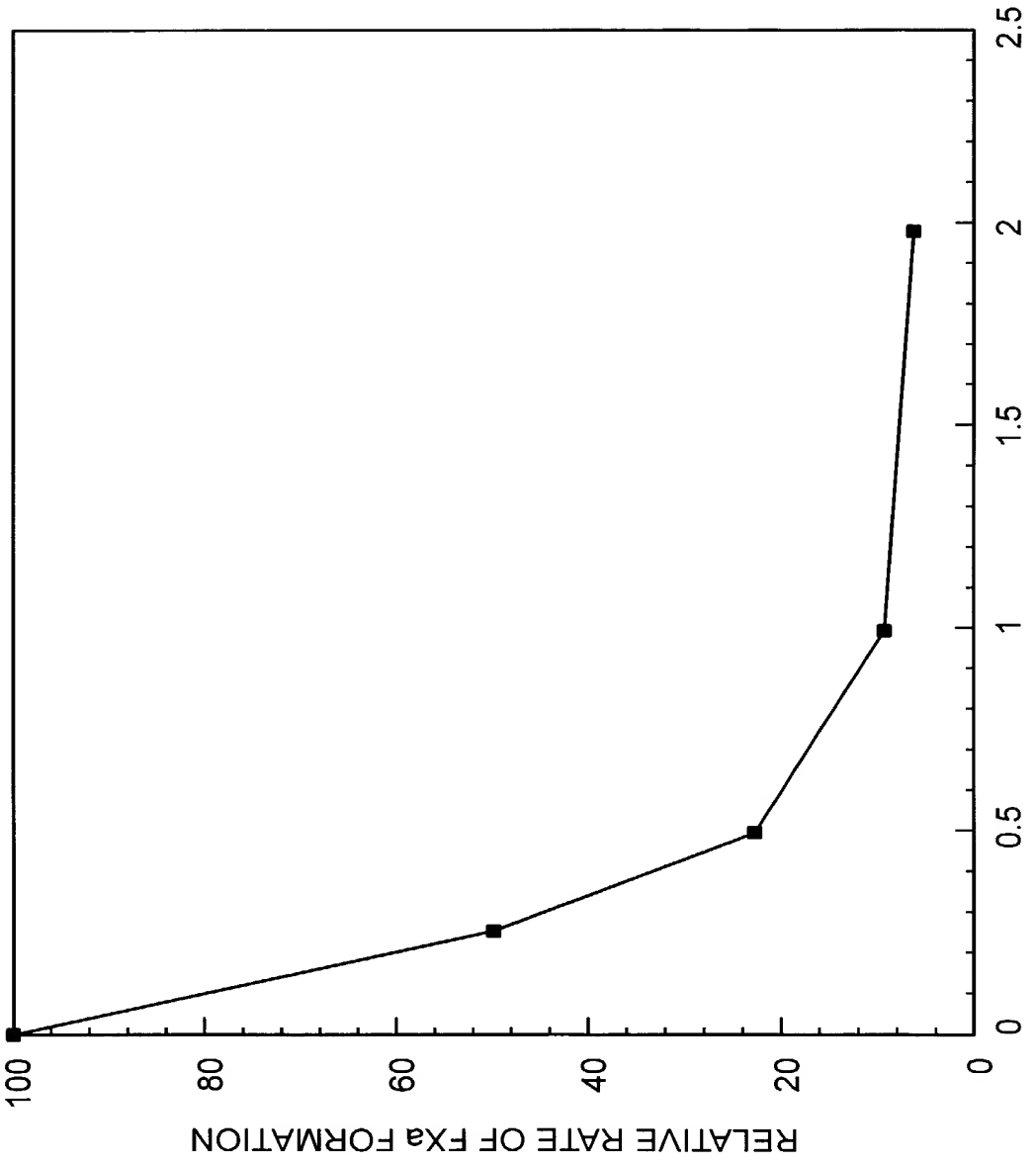
RELATIVE RATE OF FXa FORMATION
100
80
60
40
20
0
0
0.5
1
1.5
2
2.5
H36.D2/rhTF MOLAR RATIO

FIG. 6A

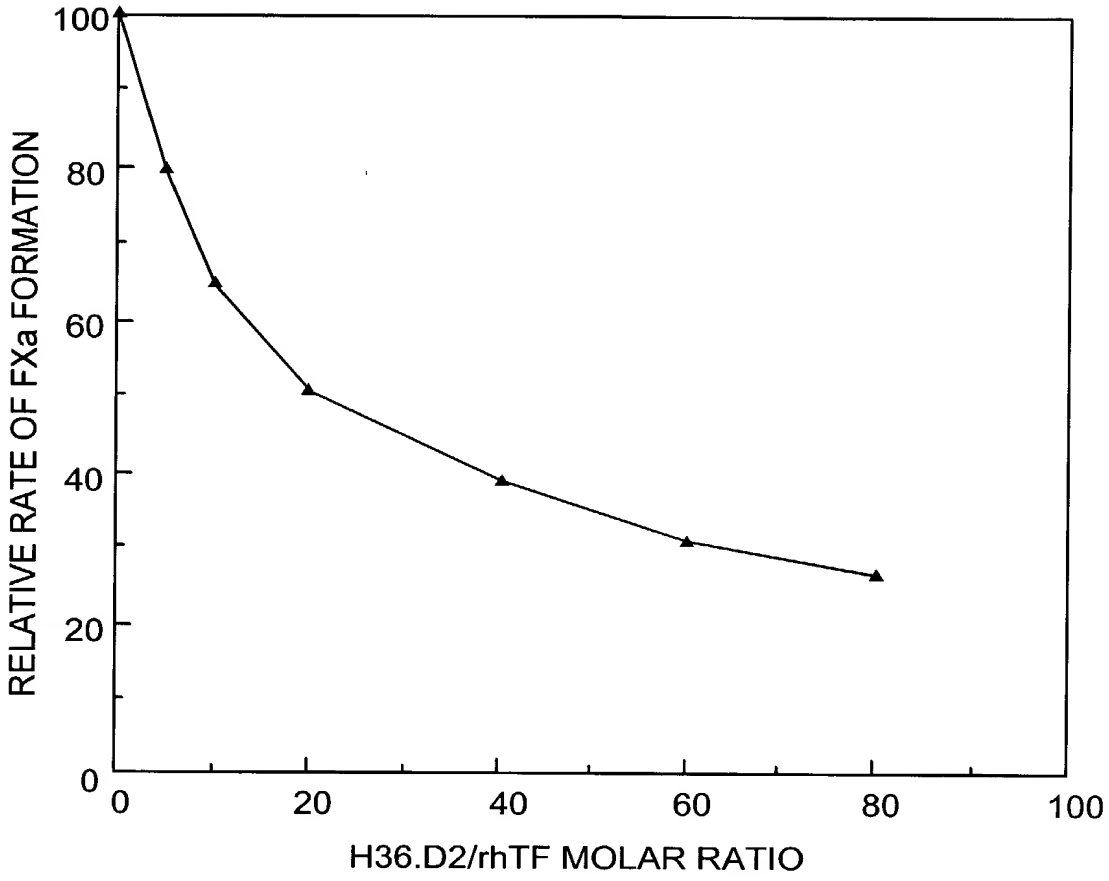
100
80
60
40
20
0
RELATIVE RATE OF FXa FORMATION
0
20
40
60
80
100
H36.D2/rhTF MOLAR RATIO

FIG. 6B

H36.D2 CONCENTRATION (ng)	% INHIBITION CELLS (TF/FVII) AND H36.D2 PREINCUBATED PRIOR TO FX ADDITION	% INHIBITION FX AND H36.D2 ARE ADDED SIMULTANEOUSLY TO CELLS (TF/FVII)
0	0	0
50	88	nd
100	92	nd
200	97	nd
800	nd	76
1600	nd	78
3200	nd	92

FIG. 7

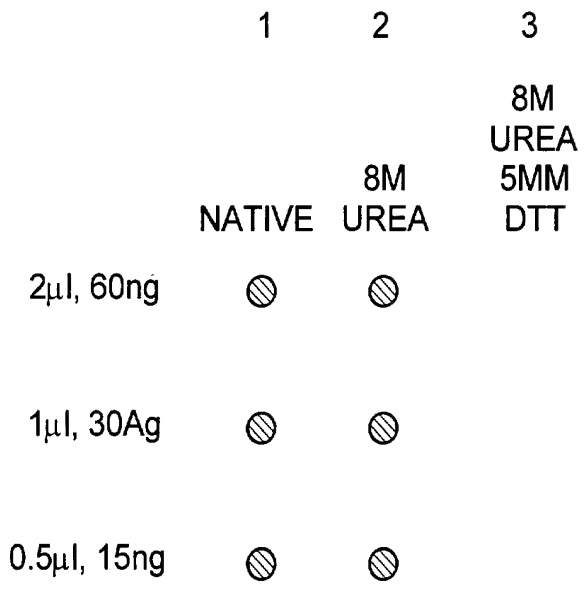

FIG. 8A

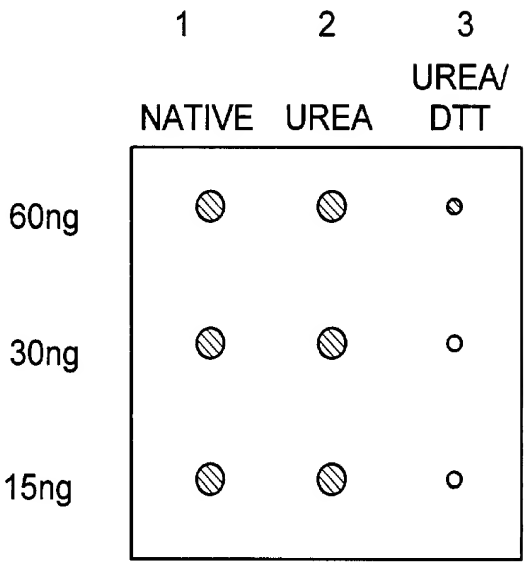

FIG. 8B

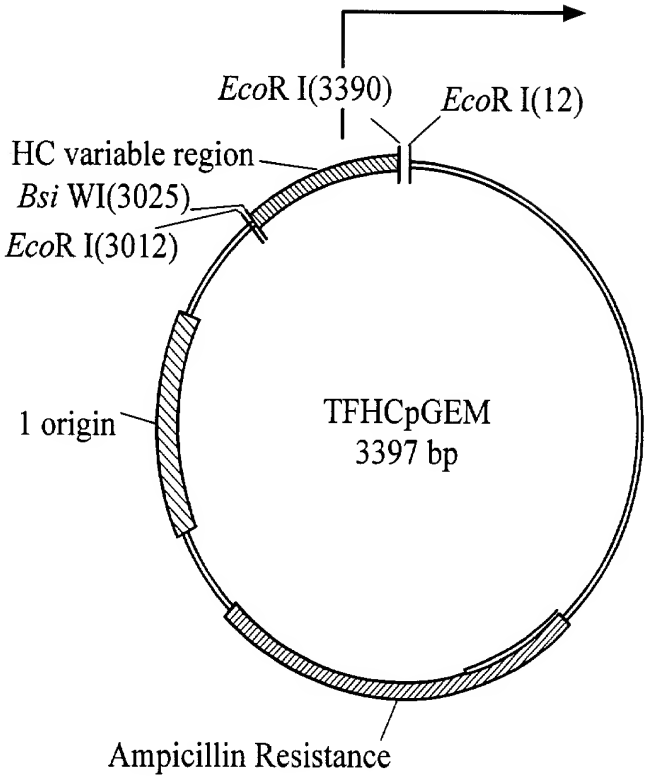
EcoR I(3390)
EcoR I(12)
HC variable region
Bsi WI(3025)
EcoR I(3012)
1 origin
TFHCpGEM
3397 bp
Ampicillin Resistance

FIG. 9A

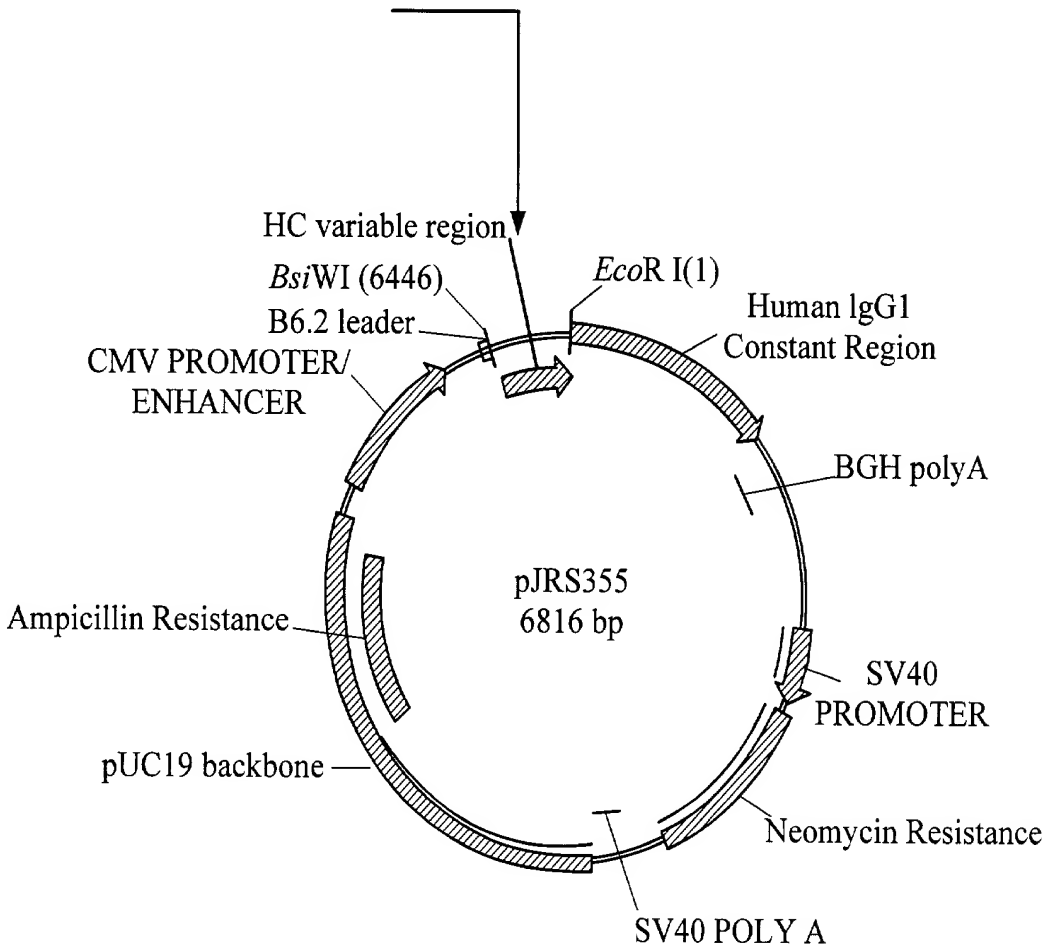
HC variable region
BsiWI (6446)
B6.2 leader
EcoR I(1)
Human lgG1
Constant Region
CMV PROMOTER/
ENHANCER
BGH polyA
pJRS355
6816 bp
Ampicillin Resistance
SV40
PROMOTER
pUC19 backbone
Neomycin Resistance
SV40 POLY A

FIG. 9B

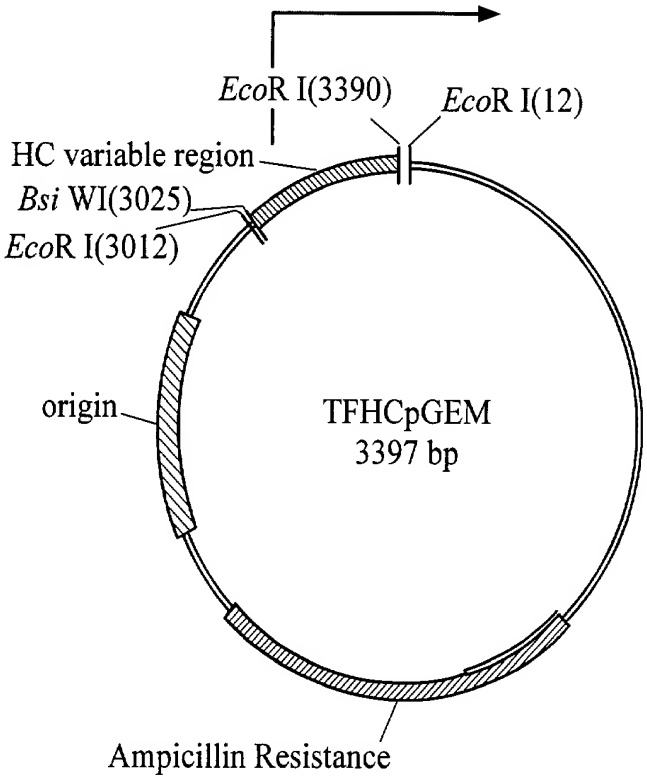

FIG. 9C

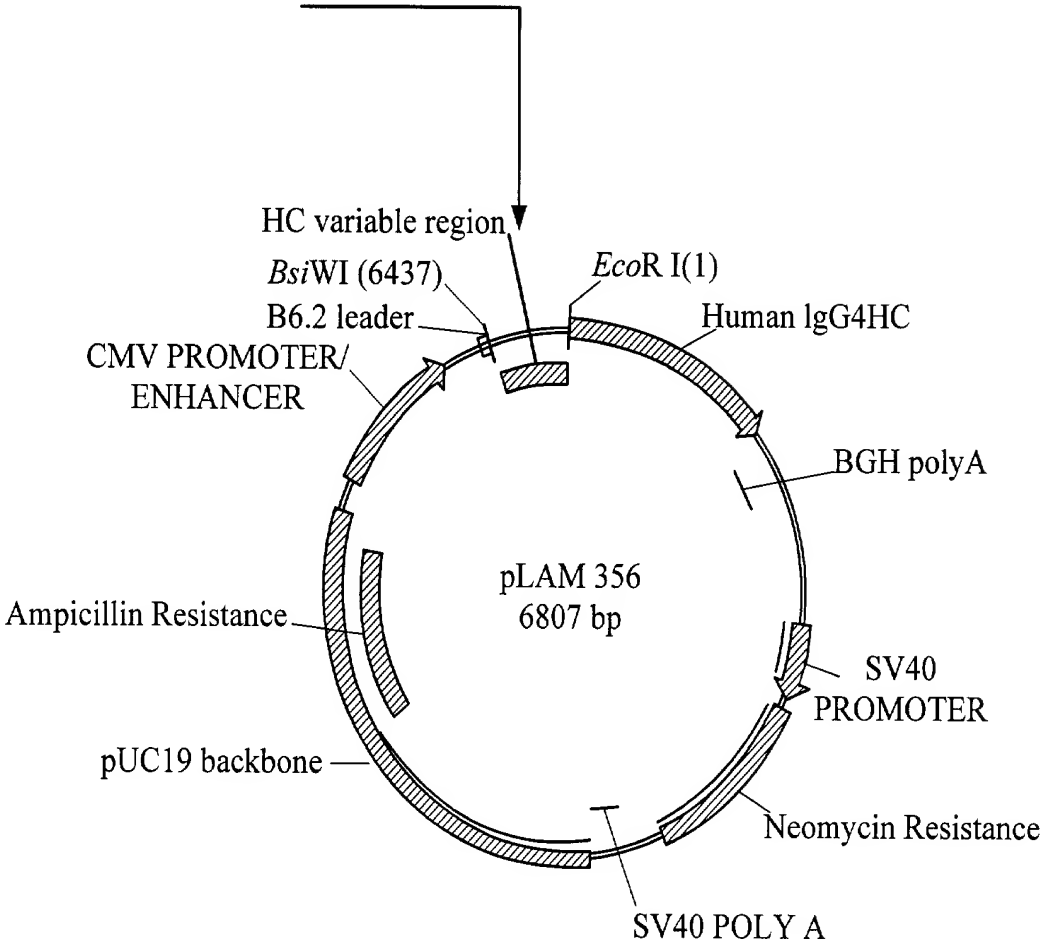

FIG. 9D

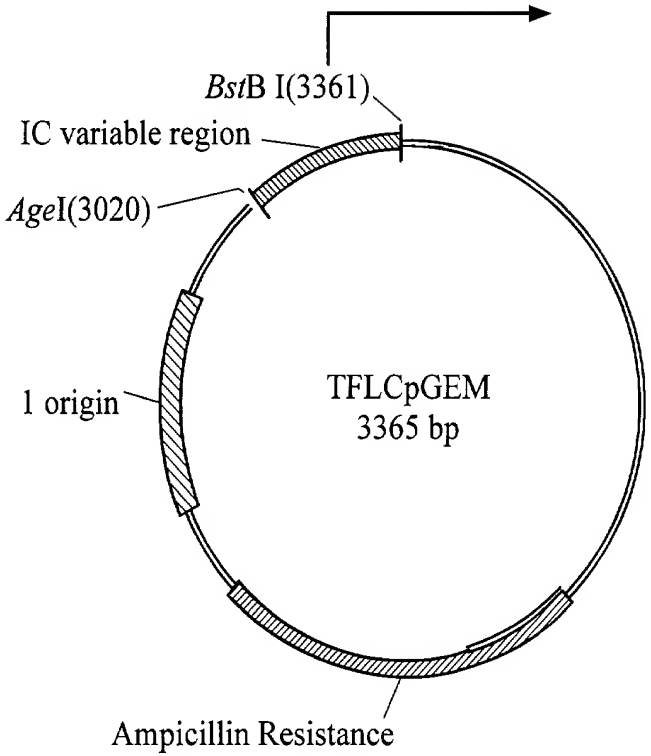

FIG. 10A

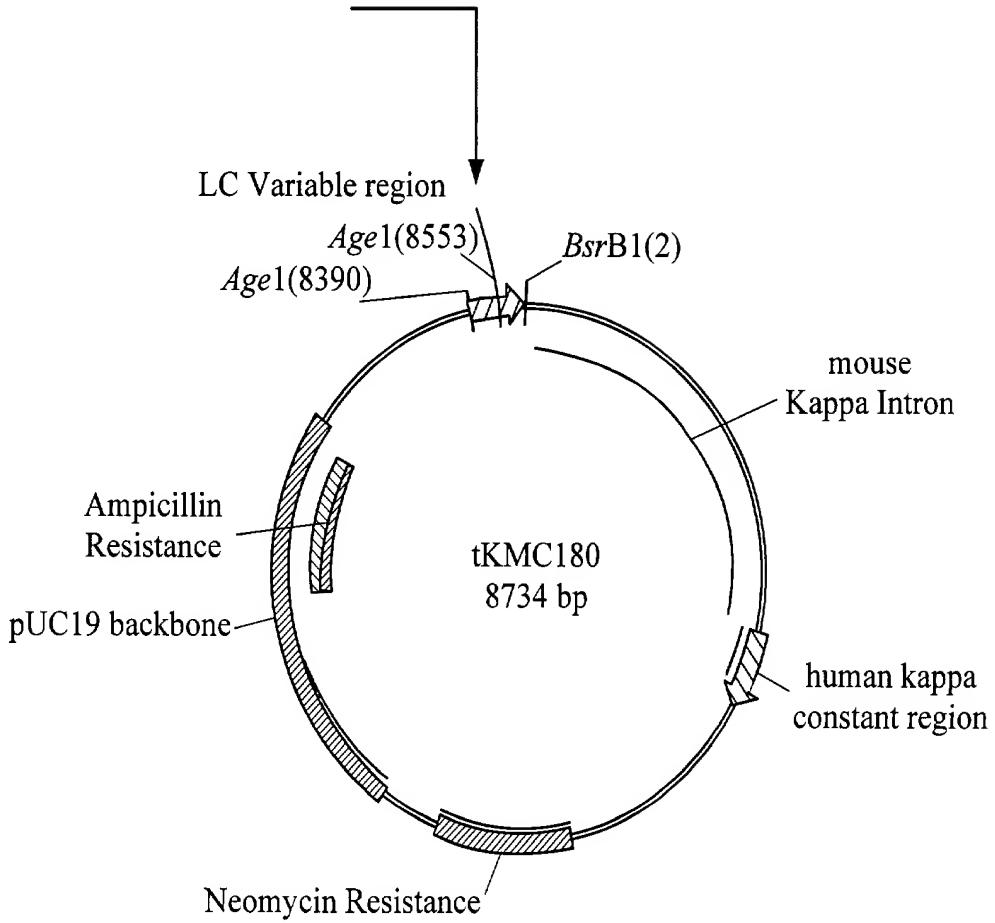

FIG. 10B

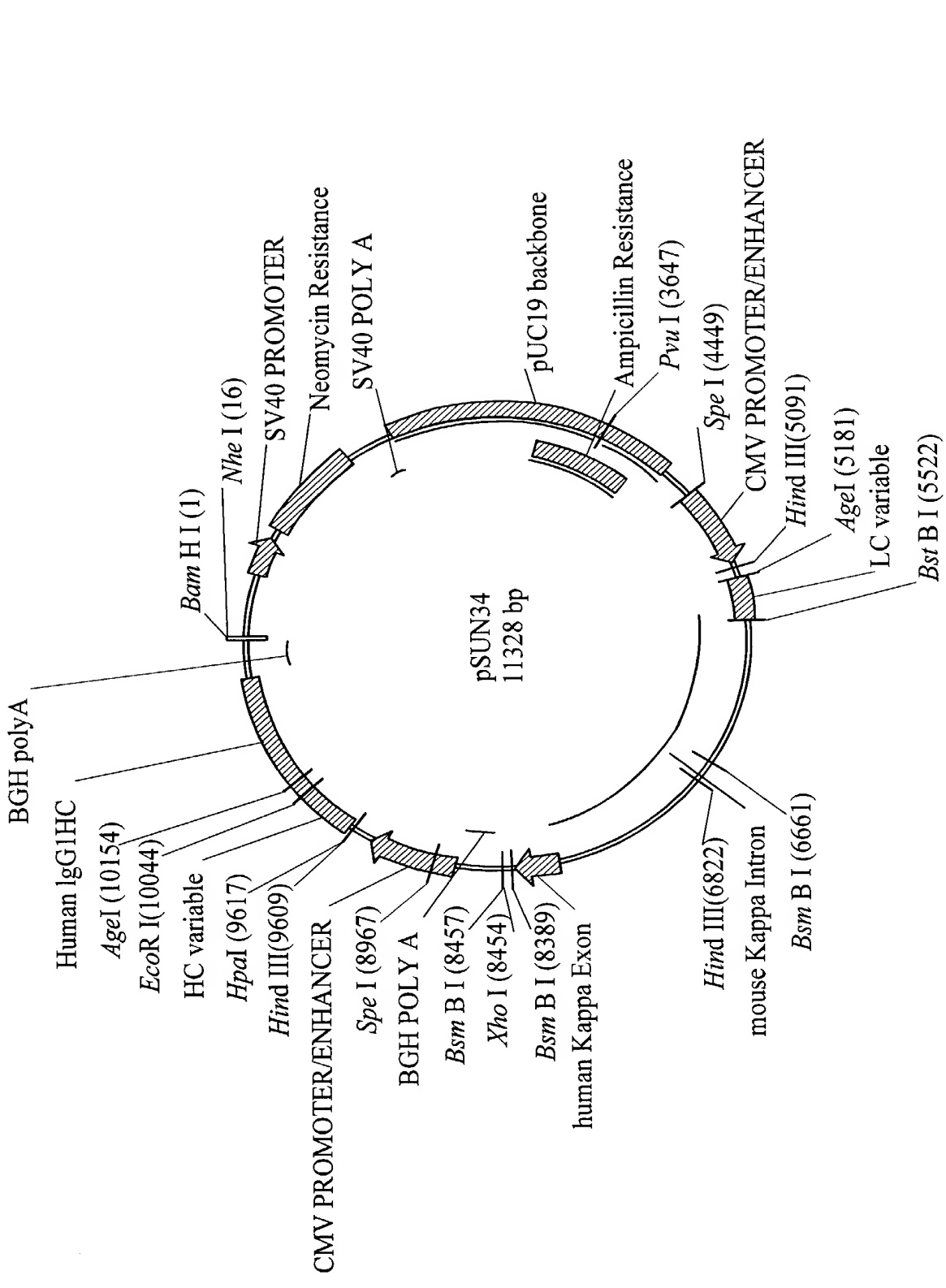
pSUN34
11328 bp
Bam H I (1)
Nhe I (16)
SV40 PROMOTER
Neomycin Resistance
SV40 POLY A
pUC19 backbone
Ampicillin Resistance
Pvu I (3647)
Spe I (4449)
CMV PROMOTER/ENHANCER
Hind III(5091)
AgeI (5181)
LC variable
Bst B I (5522)
Bsm B I (6661)
mouse Kappa Intron
Hind III(6822)
human Kappa Exon
Bsm B I (8389)
Xho I (8454)
Bsm B I (8457)
BGH POLY A
Spe I (8967)
CMV PROMOTER/ENHANCER
Hind III(9609)
HpaI (9617)
HC variable
EcoR I(10044)
AgeI (10154)
Human lgG1HC
BGH polyA

FIG. 11

Humanization of anti-Tissue Factor Antibody cH36

Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions

Light Chain (LC) FR Sequences

FR1 (23 AA) 1–23	FR2 (14 AA) 35–47	FR3 (32 AA) 57–86	FR4 (10 AA) 98–107	Names
DIQMTQSPA**S**QSASLGESV**T**ITC	**W**YQQKPGKSPQL**I**Y	**G**VP**S**RFSGSGSGT**K**FSFKISSLQ**A**EDFVN**Y**YC	**F**GAGTKLEL**K**	cH36-LC
DIQMTQSPASQSASLGESVTITC	WYQQKPGKSPQLIY	GVPSRFSGSGSGTKFSFKISSLQAEDFVNYYC	FGAGTKLEIK	LC-03
DIQMTQSPASQSASLGESVTITC	WYLQKPGKSPQLIY	GVPS FSGSGSGTKFSFKISSLQAEDFVNYYC	FGAGTKLEIK	LC-04
DIQMTQSPASLSASVGDRVTITC	WYLQKPGKSPQLIY	GVPSRFSGSGSGTKFSFKISSLQAEDFVNYYC	FGQGTKLEIK	LC-05
DIQMTQSPASQSASLGESVTITC	WYLQKPGKSPQLIY	GVPSRFSGSGSGTKFSFKISSLQAEDFVNYYC	FGQGTKLEIK	LC-06
DIQMTQSPASQSASLGESVTITC	WYLQKPGKSPQLIY	GVPSRFSGSGSGTDFSFTISSLQPEDFVNYYC	FGQGTKLEIK	LC-07
DIQMTQSPASQSASLGESVTITC	WYLQKPGKSPQLIY	GVPSRFSGSGSGTDFSFTISSLQPEDFATYYC	FGQGTKLEIK	LC-08
DIQMTQSPASLSASVGDRVTITC	WYLQKPGKSPQLIY	GVPSRFSGSGSGTDFSFTISSLQPEDFATYYC	FGQGTKLEIK	LC-09
DIQMTQSPASLSASVGDRVTITC	WYLQKPGKSPQLIY	GVPSRFSGSGSGTDFSFTISSLQPEDFANYYC	FGQGTKLEIK	LC-10
DIQMTQSPASLSASVGDRVTITC	WYLQKPGKSPQLIY	GVPSRFSGSGSGTKFSFTISSLQPEDFANYYC	FGQGTKLEIK	LC-11
DIQMTQSPASLSASVGDRVTITC	WYLQKPGQSPQLIY	GVPSRFSGSGSGTKFSFTISSLQPEDFANYYC	FGQGTKLEIK	LC-12

Position markers as printed: 1, 10, 20 (FR1); 35, 47 (FR2); 57, 60, 70, 80, 86 (FR3); 98, 107 (FR4).

FIG. 12A

Light Chain CDR Sequnces of cH36

CDR1 (11 AA)
24 – 34
L A S Q T I D T W L A

FIG. 12B

CDR2 (7 AA)
50 – 56
A A T N L A D

FIG. 12C

CDR3 (9 AA)
89 – 97
Q Q V Y S S P F T

FIG. 12D

Sequences of Partially and Fully Humanized Heavy Chain (LC) Variable Regions

Heavy Chain (HC) FR Sequences

FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA)	FR4 (11 AA)	Names
1 10 20 29	36 44	67 75 85 95	107 117	
EIQLQQSGP**E**LVKPGASV**Q**VSCKTSGYS**F**T	**W**VRQSHGK**S**LEWIG	**K**ATLTVDK**S**STTAFMHLN**S**LTSDDSAVY**F**CAR	**W**GQGTTLTVS**S**	cH36-HC
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSHGKSLEWIG	KATLTVDKSSTTAFMHLNSLTSDDSAVYFCAR	WGQGTTVTVSS	HC-01
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLTVDKSSTTAFMHLNSLTSDDSAVYFCAR	WGQGTTVTVSS	HC-02
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLTVDKSSTTAFMHLNSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-03
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLTVDKSSTTAFMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-04
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-05
QMQLQQSGGELVKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-06
QIQLVQSGGELVKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-07
QIQLVQSGGEVKKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-08
QIQLVQSGGEVKKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-08R1
QIQLVQSGPEVKKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-11
QIQLVQSGPELKKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-12
QIQLVQSGPELVKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-09
QIQLVQSGPEVVKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAYMELSSLRSEDTAVYFCAR	WGQGTTVTVSS	HC-10

FIG. 13A

Heavy Chain CDR Sequnces

CDR1 (5 AA)	CDR2 (17 AA)	CDR3 (8AA)	Names
31 35	50 66	99 106	
D Y N V Y	*Y I D P Y N G I T I Y D Q N F K G*	*D V T T A L D F*	***cH36***
31 35	50 66	99 106	
D Y N V Y	*Y I D P Y N G I T I Y D Q N L K G*	*D V T T A L D F*	***HC-08***

FIG. 13B FIG. 13C FIG. 13D

hOAT (IgG1) CONSTANT REGIONS SEQUENCES

SEQUENCES OF LC CONSTANT:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH

KVYACEVTHQGLSSPVTKSFNRGEC

FIG. 14A

SEQUENCES OF HC CONSTANT:

EFASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC

NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSSHEDPEVKFNWYVDGVEV

HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL

VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIG. 14B

hFAT (IgG1) CONSTANT REGION SEQUENCES

SEQUENCES OF LC CONSTANT:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK

HKVYACEVTHQGLSSPVTKSFNRGEC

FIG. 15A

SEQUENCES OF HC CONSTANT:

EFASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY

TCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV

EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL

TCLVKGFYPSDDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

FIG. 15B